

FIG. 11

Signal to noise ratio of ELISA
reading using phospho-Serine/threonine
phenylalanine antibody.

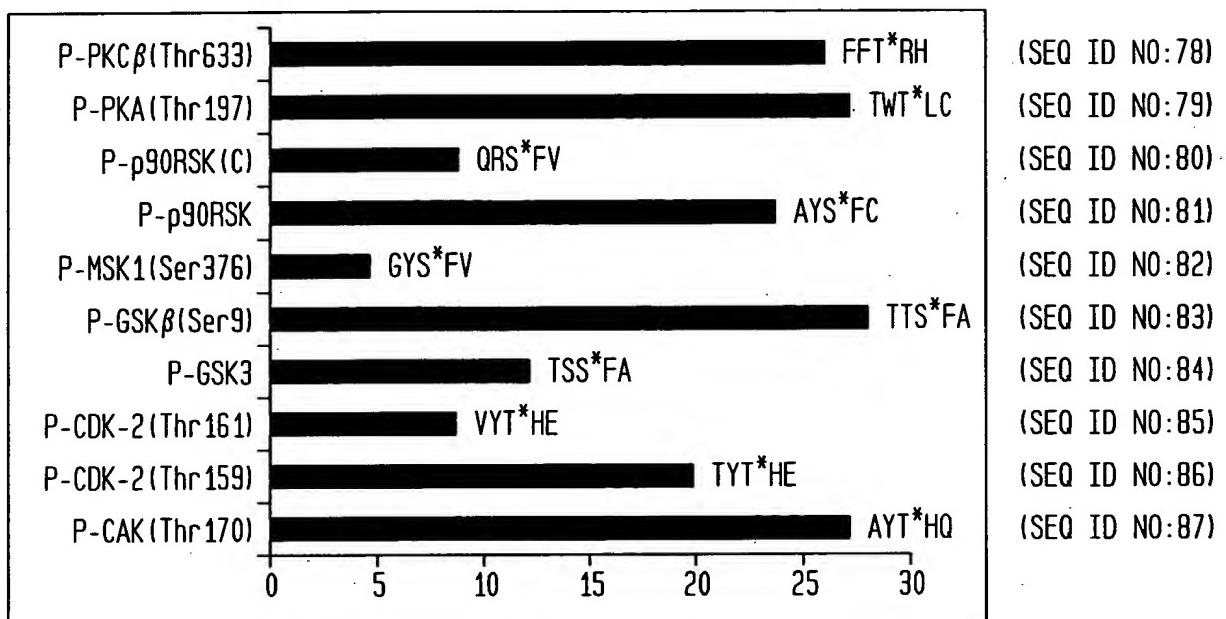
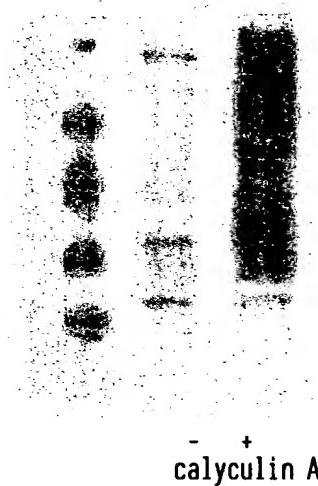


FIG. 12

Western analysis of calyculin
A-treated A431 cells using phospho-
Serine/phenylalanine substrates antibody.



BEST AVAILABLE

FIG. 7

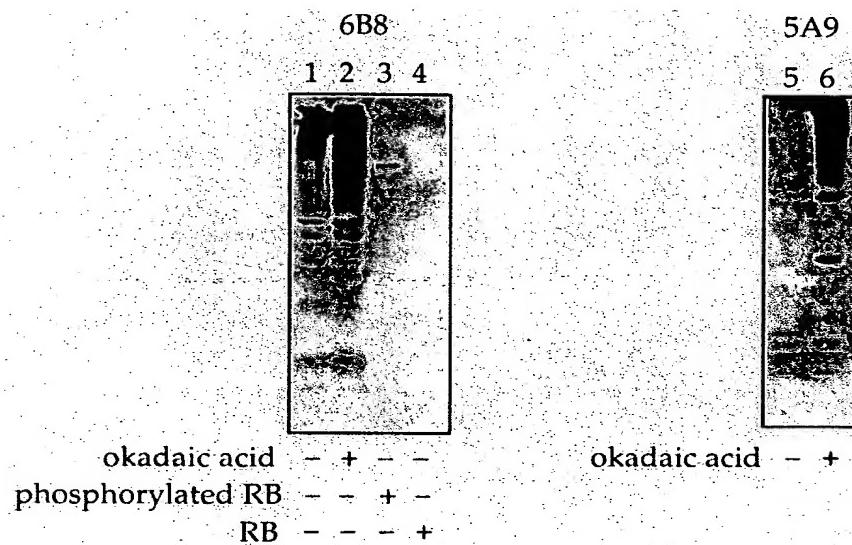
Western analysis of
calyculin A-treated A431 cells
using Phospho-Akt Substrate Anti-
body.



- +
calyculin A

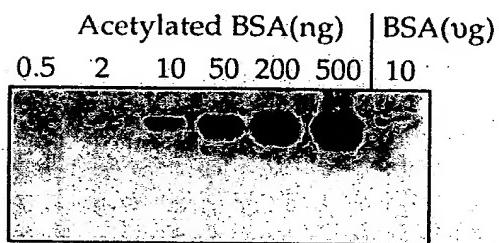
BEST AVAILABLE COPY

FIG. 4B



BEST AVAILABLE COPY

FIG. 5A



BEST AVAILABLE COPY

FIG. 5B

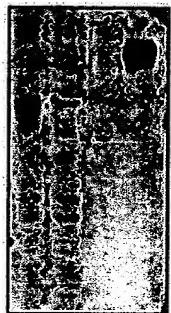


FIG. 5C



FIG. 5D



FIG. 3B

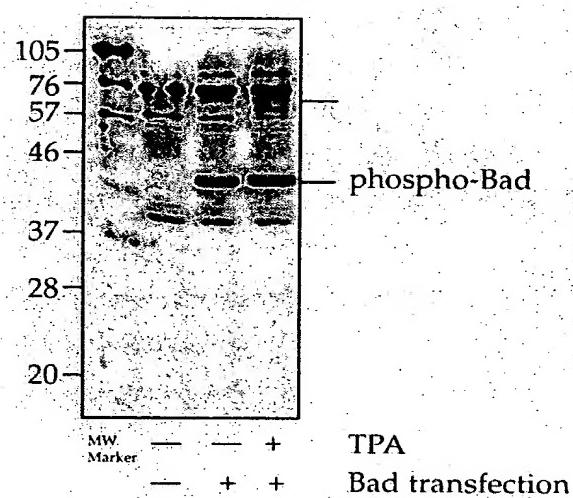
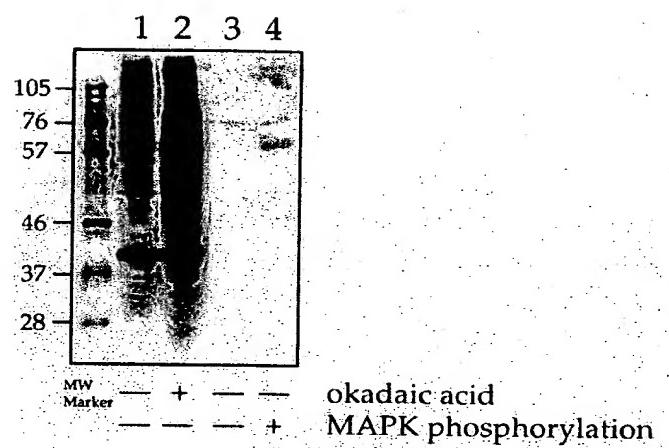


FIG. 2B



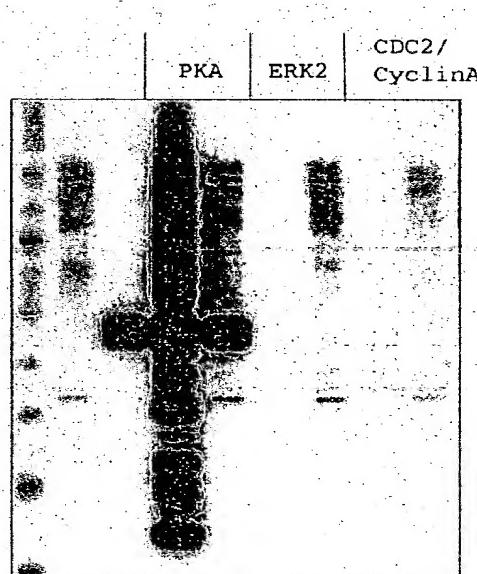


- + calyculin A

FIG. 9

Western analysis of
calyculin A-treated A431 cells
using Phospho-PKA Substrate
Antibody.

BEST AVAILABLE COPY



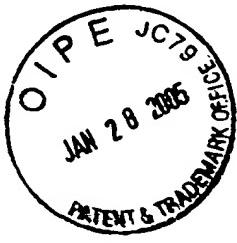
+ - + + - + -
- - - + - - -

Cell Extracts
PKI

FIG. 10

Western analysis of
A431 cell extracts

FIG. 1A



PEPTIDE	SEQUENCE	ANTIBODY DILUTIONS
		6.00E+03 1.00E+03 5.00E+04 1.00E+05 5.00E+05 1.00E+06
Thr'	X-X-X-X-Thr-X-X-X-X-Cys	1.92 1.32 0.54 0.34 0.07 0.04
Ser-Thr	X-X-X-X-Ser/Thr-X-X-X-X-Cys	0.11 0.05 0.01 0.00 0.01 0.00
Theanine' mix	18 phospho-Thr peptide	1.84 1.13 0.40 0.26 0.10 0.07
Serine' mix	38 phospho-Ser peptide	0.12 0.04 0.02 0.02 0.02 0.01
Akt-Thr180-P	Ile-Lys-Asp-Gly-Ala-Thr-Met-Lys-Thr-Pro-Gly-Thr-Thr-Pro	1.18 0.65 0.24 0.13 0.03 0.01
(SEQ ID NO: 1)		
AP1-Thr689-P	Asp-Ala-Ala-Val-Thr-Pro-Lys-Lys-Ala-Ala-Thr-Ser-Lys-Cys	0.14 0.03 0.01 0.01 0.01 0.00
(SEQ ID NO: 2)		
C3-P	Asp-Thr-Gln-Lys-Ala-Ala-Thr-Pro-Gly-Thr-Pro-Pro-Gly	1.71 1.13 0.38 0.22 0.03 0.02
(SEQ ID NO: 3)		
CAK-Thr167-P	His-Gln-Val-Val-Thr-Ala-Tyr-Tyr-Ala-Cys	1.77 1.15 0.41 0.27 0.06 0.03
(SEQ ID NO: 4)		
GAMIV-Thr188-P	His-Gln-Val-Leu-Met-Lys-Thr-YVal-Gly-Gly	1.79 1.36 0.63 0.40 0.09 0.05
(SEQ ID NO: 5)		
CD22-Thr167-P	Ile-Pro-Ile-Ala-Val-Tyr-Thr-His-Glu-Va-Lys-Val-Thr-Leu-Cys	1.02 0.56 0.14 0.08 0.03 0.01
(SEQ ID NO: 6)		
CD162-Thr169-P	Gly-Val-Pro-Val-Ala-Thr-Tyr-Thr-His-Glu-Va-Lys-Thr-Leu-Cys	1.88 1.78 0.51 0.44 0.08 0.04
(SEQ ID NO: 7)		
D708K-Thr389-P	Asn-Gln-Val-Phe-Leu-Gly-Pro-Thr-Tyr-Val-Ala-Pro-Lys-Lys-Cys	1.89 1.44 0.62 0.39 0.08 0.01
(SEQ ID NO: 8)		
PCcalpha-P	Lys-Glu-His-Met-Ala-Asp-Gly-Va-Lys-Thr-Ala-Thr-Pro-Gly	1.62 1.63 0.84 0.58 0.15 0.08
(SEQ ID NO: 9)		
E882P	Asp-His-Thr-Gly-Phe-Leu-Thr-Glu-Tyr-Va-Ala-Thr-Ala-Pro-Tyr-Cys	1.56 1.18 0.51 0.30 0.07 0.04
(SEQ ID NO: 10)		
Myc Ser58/62-P	Glu-Lys-Leu-Pro-Thr-Pro-Pro-Lys-Ser-Pro-Ser-Ala-Ala-Ser-Cys	0.11 0.06 0.03 0.02 0.02 0.02
(SEQ ID NO: 11)		
R38-2P	Leu-Ala-Alp-His-Thr-Ala-Asp-Glu-Met-Thr-Gly-Tyr-Val-Ala-Thr-Ala-Cys	0.54 0.30 0.08 0.06 0.04 0.02
(SEQ ID NO: 12)		
INK-2P	Ser-Phe-Met-Met-Thr-Pro-Pro-Tyr-YVal-Val-Val-Thr-Ala-Tyr-Ala-Cys	1.49 0.44 0.12 0.07 0.03 0.02
(SEQ ID NO: 13)		

FIG. 1B

PEPTIDE SEQUENCE	phospho-Thr Reactivity	
XXXXXXS*XXXXXX	—	
XXXXY*XXXX	—	
XXXXXPXS*/T*PXR/KXXX (SEQ ID NO:14)	++	
XXXXRSXS*XPXXXX (SEQ ID NO:15)	—	
XXXXRSXSXPXXXX (SEQ ID NO:16)	—	
XXXXXPXS*/T*PXXXXX (SEQ ID NO:17)	++	
XXXXXPXS/TPXXXXX (SEQ ID NO:18)	—	
XXXXXT*XXXXXX	+++	
XXXXXXS/TXXXXXX	—	
21 phospho-Thr peptides mixture	+++	
38 phospho-Ser peptides mixture	—	
30 phospho-Tyr peptides mixture	—	
 NEB LIBRARY		
X-X-X-X-D/E-X-X-S*-T*-X-X-X-X-X-C (SEQ ID NO:19)	+++	
X-X-X-X-X-X-S*/T*-D/E-D/E-D/E-X-X-X (SEQ ID NO:20)	++	
X-X-X-X-F-X-X-F-S*/T*-F/Y-X-X-X-X-C (SEQ ID NO:21)	+++	
X-X-X-X-R/K-X-S*/T*-X-X-X-X-X-X-C (SEQ ID NO:22)	+++	
X-X-X-R/K-X-X-S*/T*X-X-X-X-X-X-C (SEQ ID NO:23)	+++	
X-X-X-X-X-X-S*/T*-F/I/M-X-X-X-X-X-C (SEQ ID NO:24)	+++	
X-X-X-X-X-X-S*/T*-F/I-X-X-X-X-X-C (SEQ ID NO:25)	+++	
X-X-X-X-X-X-S*/T-P-X-X-X-X-X-C (SEQ ID NO:26)	++	
X-X-X-X-X-T*-X-X-X-X-X-X-C	+++	
X-X-X-X-X-P-X-S*/T*-P-X-X-X-X-X-C (SEQ ID NO:27)	++	
X-X-X-X-X-X-S/T-X-X-X-X-X-X-C (SEQ ID NO:28)	—	
X-X-X-X-X-P-X-S*/T*-P-X-R/K-X-X-X-C (SEQ ID NO:29)	++	
 ANTIBODY REACTIVITY		
ELISA O.D.		
+++	<i>very strong</i>	> 2
++	<i>strong</i>	1 - 2
+	<i>weak</i>	0.2 - 1
-	<i>very little</i>	< 0.2

FIG. 1D

FIG. 2A

PEPTIDE	SEQUENCE	1.00E+03	5.00E+03	1.00E+04	5.00E+04	1.00E+05	5.00E+05	1.00E+06
PSSAP	XXXXX-Pro-X-Ser-Thr-Pro-XXXXX-Cys (SEQ ID NO: 27)	1.82	1.87	1.74	1.40	0.70	0.35	0.08
Threonine mix	18 phospho-Thr peptide mix	1.97	1.37	0.87	0.36	0.13	0.07	0.05
Ser/Thr	X-X-X-X-X-Ser/Thr-X-X-X-X-Cys (SEQ ID NO: 28)	0.14	0.03	0.01	0.00	0.00	0.00	0.00
RB_Thr373-P	Val-Ile-Pro-Pro-His-Thr'-Pro-Val-Ala-Thr-Met-Asn-Thr-Cys (SEQ ID NO: 30)	2.07	2.17	1.70	1.20	0.48	0.18	0.03
MKKG-Thr-P	Ser-Yal-Ala-Lys-Thr'-Met-Asp-Ala-Gly-Cys (SEQ ID NO: 31)	0.06	0.04	0.01	0.00	0.00	0.00	0.00
PKCalpha-P	Lys-Glu-His-Met-Met-Asp-Gly-Vel-Thr-Ary-Thr'-Phe-Cys (SEQ ID NO: 9)	0.05	0.02	0.01	0.00	0.01	0.00	0.00
p70_S6K_Thr389	Asn-Gln-Vel-Phe-Leu-Gly-Phe-Thr'-Tyr-Val-Ala-Pro-Lys-Lys-Cys (SEQ ID NO: 8)	0.11	0.05	0.01	0.00	0.01	0.00	0.00
cock4-Thr172-P	Arg-Ile-Tyr-Ser-Tyr-Gln-Met-Ala-Ileu-Thr'-Pro-Val-Val-Val-Lys-Cys (SEQ ID NO: 32)	2.07	2.21	2.01	1.55	0.89	0.31	0.07

FIG. 3A

PEPTIDE	SEQUENCE				ANTIBODY DILUTIONS	
		1.00E+03	5.00E+03	1.00E+04	5.00E+05	1.00E+05
14-3 BM-P	XX-XX-Ag-Ser-X-Ser-X-Pro-XX-XX-Cys					
	(SEQ ID NO:33)	2.41	2.15	1.49	1.15	0.44
14-3 BM	XX-XX-Ag-Ser-X-Ser-X-Pro-X-XX-X-Cys					
	(SEQ ID NO:34)	0.07	0.03	0.02	0.03	0.02
CDC25-Ser216-P	Gly-Leu-Tyr-Arg-Ser-Pro-Ser-Met-Pro-Glu-Asn-Leu-Asn-Arg-Cys					
	(SEQ ID NO:35)	2.35	2.08	1.49	1.05	0.33
CDC25-Ser218	Gly-Leu-Tyr-Arg-Ser-Pro-Ser-Met-Pro-Glu-Asn-Leu-Asn-Arg-Cys					
	(SEQ ID NO:36)	0.05	0.02	0.03	0.03	0.04
Bad-Ser112-P	Thr-Arg-Ser-Arg-His-Ser-Ser-Tyr-Pro-Ala-Gly-Thr-Glu-Glu-Cys					
	(SEQ ID NO:37)	1.59	0.43	0.10	0.03	0.01
Bad-Ser112	Thr-Arg-Ser-Arg-His-Ser-Ser-Tyr-Pro-Ala-Gly-Thr-Glu-Glu-Cys					
	(SEQ ID NO:38)	0.00	0.00	0.00	0.00	0.00
Bad-Ser138	Phe-Arg-Gly-Arg-Ser-Arg-Ser-Ala-Pro-Pro-Asn-Leu-Trp-Ala-Cys					
	(SEQ ID NO:39)	0.03	0.00	0.00	0.00	0.00
Bad-Ser138-P	Phe-Arg-Gly-Arg-Ser-Arg-Ser-Ala-Pro-Pro-Asn-Leu-Trp-Ala-Cys					
	(SEQ ID NO:40)	3.25	1.86	0.73	0.51	0.07

FIG. 4A

PEPTIDE	SEQUENCE	MONOCLONAL ANTIBODIES	
		6B8	5A9
Ser/ThrPro-P	X-X-X-X-X-Ser ^r /Thr-Pro-X-X-X-X-X-Cys (SEQ ID NO:26)	1.774	0.731
ProXSer/ThrPro-P	X-X-X-X-Pro-X-Ser ^r /Thr ^r -Pro-X-X-X-X-Cys (SEQ ID NO:27)	0.924	0.766
ProXSer/ThrPro-P	X-X-X-X-X-Pro-X-Ser/Thr-Pro-X-X-X-X-X-Cys (SEQ ID NO:41)	0.02	0.063
ProXSer/ThrProArg-P	X-X-X-X-Pro-X-Ser ^r /Thr ^r -Pro-X-Arg/Lys-X-X-Cys (SEQ ID NO:42)	1.955	1.275
Thr-P	X-X-X-X-X-Thr ^r X-X-X-X-X-X-Cys	0	..
Ser-P	X-X-X-X-X-Ser ^r X-X-X-X-X-X-Cys	0.031	0.088
Ser/Thr	X-X-X-X-X-Ser/Thr-X-X-X-X-X-X-Cys	0.021	0.066
Tyr-P	X-X-X-X-X-Tyr ^r X-X-X-X-X-X-Cys	0.023	0.072
Rb (Ser795)-P	Ser-Pro-Tyr-Lys-Phe-Pro-Ser-Ser ^r -Pro-Leu-Arg-Ile-Pro-Gly-Cys (SEQ ID NO:43)	0.032	0.124
Rb (Thr373)-P	Val-Ile-Pro-Pro-His-Thr ^r -Pro-Val-Arg-Thr-Val-Met-Asn-Thr-Cys (SEQ ID NO:30)	3.336	3.503
Rb (Thr373)	Val-Ile-Pro-Pro-His-Thr-Pro-Val-Arg-Thr-Val-Met-Asn-Thr-Cys (SEQ ID NO:44)	0.02	0.073

FIG. 6

Signal to noise ratio of ELISA
readings using phospho-Akt substrate
antibody.

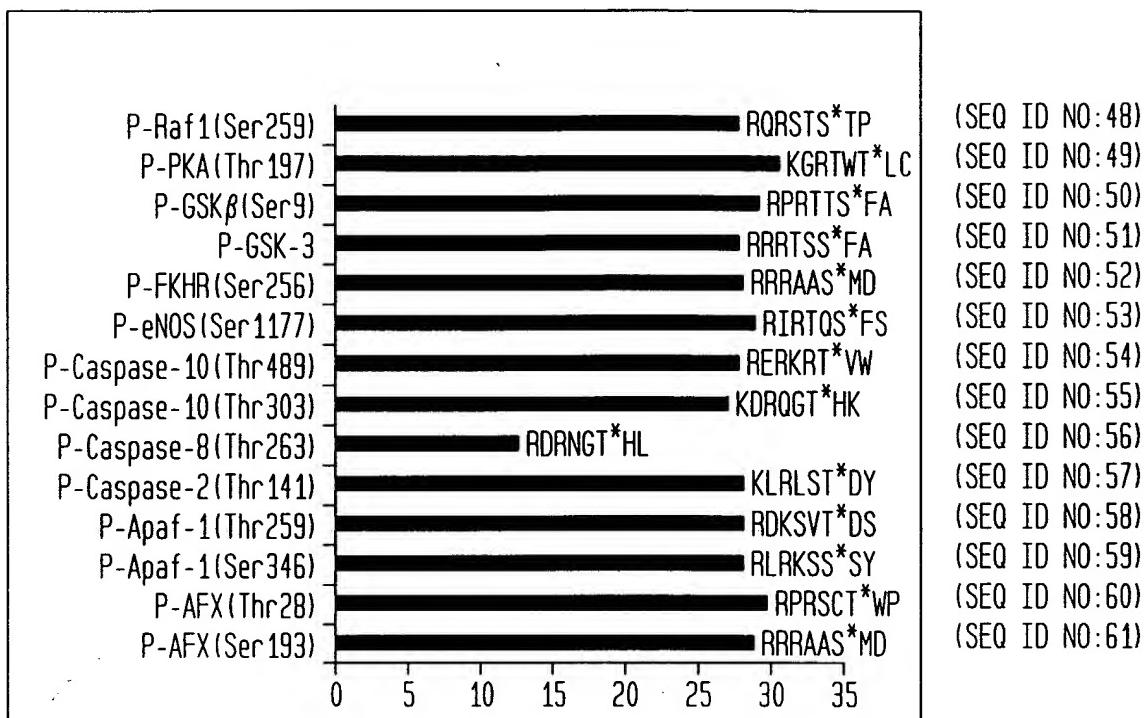


FIG. 8

Signal to noise ratio of ELISA
reading using phospho-PKA substrates
antibody.

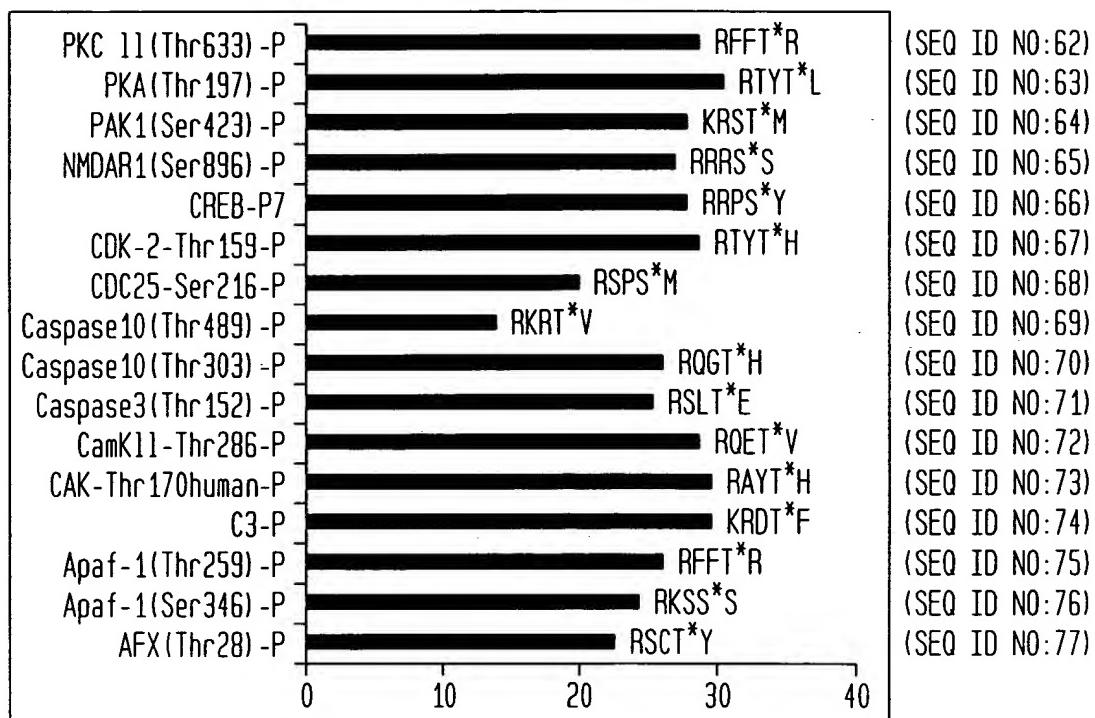




FIG. 1A

PEPTIDE	SEQUENCE	ANTIBODY DILUTIONS
Thr*	XXXXXXXX-Thr-XXXXXX-Cys	1.00E+03 6.00E+03 1.00E+04 5.00E+04 1.00E+05 5.00E+05 1.00E+06
Thr*	XXXXXXXX-X-Ser(Thr)XXX-XXXX-Cys	1.92 1.32 0.54 0.34 0.07 0.04 0.02
Ser-Thr	XXXXXXXX-X-Ser(Thr)XXX-XXXX-Cys	0.11 0.05 0.01 0.00 0.01 0.00 0.00
Threonine* mix	18 phospho-Thr peptide	1.84 1.13 0.40 0.28 0.10 0.07 0.05
Serine* mix	38 phospho-Ser peptide	0.12 0.04 0.02 0.02 0.02 0.01 0.00
AKT-Thr608-P	Ile-Lys-Asp-Gly-Ala-Thr-Met-Lys-Thr-Pro-Gly-Thr-Pro	1.18 0.65 0.24 0.13 0.03 0.01 0.00
(SEQ ID NO: 1)		
APP1-Thr688-P	Asp-Ala-Ala-Ala-Ala-Thr-Pro-Lys-Lys-Arg-His-Leu-Ser-Lys-Cys	0.14 0.03 0.01 0.01 0.01 0.01 0.00
(SEQ ID NO: 2)		
C3-P	Ile-Thr-Gln-Ile-Lys-Arg-Asn-Thr-Pro-Gly-Thr-Pro-Gly-Cys	1.71 1.13 0.39 0.22 0.03 0.02 0.02
(SEQ ID NO: 3)		
GAK-Thr167-P	His-Gln-Yel-Vel-Thr-Ala-Tip-Tyr-Ala-Cys	1.77 1.15 0.41 0.27 0.06 0.03 0.01
(SEQ ID NO: 4)		
CAMIV-Thr188-P	His-Gln-Vel-Lys-Met-Lys-Thr-Vel-Gly-Cys	1.79 1.36 0.63 0.40 0.09 0.05 0.01
(SEQ ID NO: 5)		
CDK2-Thr167-P	Ile-Pro-Gln-Ala-Vel-Yer-Thr-His-Glu-Vel-Vel-Thr-Lys-Cys	1.02 0.58 0.14 0.08 0.03 0.01 0.01
(SEQ ID NO: 6)		
CDK2-Thr159-P	Gly-Vel-Pro-Vel-Ala-Thr-Tyr-Thr-His-Glu-Vel-Vel-Thr-Lys-Cys	1.88 1.79 0.51 0.44 0.08 0.04 0.02
(SEQ ID NO: 7)		
p70S6K-Thr388-P	Asn-Gln-Vel-Pho-Lys-Gly-Pro-Thr-Tyr-Val-Ala-Pro-Lys-Lys-Cys	1.99 1.44 0.62 0.39 0.08 0.04 0.01
(SEQ ID NO: 8)		
PKCalpha-P	Lys-Glu-His-Met-Asp-Gly-Vel-Thr-Thr-Arg-Thr-Pro-Cys	1.82 1.63 0.84 0.58 0.15 0.08 0.02
(SEQ ID NO: 9)		
ERG2-P	Asp-His-Thr-Gln-Pro-Lys-Thr-Gln-Tyr-Vel-Ala-Thr-Arg-Tip-Cys	1.56 1.18 0.51 0.30 0.07 0.04 0.02
(SEQ ID NO: 10)		
Myc Ser53/G2-P	Glu-Lys-Lys-Pro-Thr-Pro-Pro-Lys-Ser-Pro-Ser-Ala-Ala-Thr-Arg-Ser-Cys	0.11 0.05 0.03 0.02 0.02 0.02 0.02
(SEQ ID NO: 11)		
P38-2P	Leu-Ala-Ala-Thr-Asp-Glu-Met-Thr-Gly-Tyr-Vel-Ala-Thr-Arg-Cys	0.54 0.30 0.08 0.08 0.04 0.04 0.02
(SEQ ID NO: 12)		
JNK2P	Ser-Pro-Met-Met-Thr-Pro-Tyr-Vel-Vel-Thr-Arg-Tyr-Tyr-Ala-Cys	1.49 0.44 0.12 0.07 0.03 0.02 0.02
(SEQ ID NO: 13)		